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CHRONIC INTRAVENOUS ADMINISTRATION OF LENTINAN TO THE RHESUS MONKEY

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SUMMARY

The prolonged effects of overdosage with lentinan in the rhesus monkey are associated with foam cell reactions in lung, liver, kidney, spleen, lymph nodes and bone marrow and with varying degrees of vasculitis and associated reactions. A dose level of 0.5 mg/kg/day was without adverse effect.

INTRODUCTION

Lentinan, a polysaccharide, has been shown to have antitumour activity [1]. This paper reports on the chronic toxicity of this material, when given i.v. to rhesus monkeys for 6 months.

MATERIALS AND METHODS

30 wild-caught rhesus monkeys *(Macaca mulatta)* 2.3–3.5 kg were used. Each was housed in a wall-mounted cage, in a room maintained at a temperature of approx. 22°C in normal daylight conditions. The animals were fed a standard laboratory diet, supplemented with wholemeal bread, fresh fruit or vegetable produce, together with commercially available vitamin C and B12 supplements. Water was available at all times.

The animals were allocated to 5 groups, each of 3 males and 3 females. Lentinan was administered by i.v. injection (10 ml/min) via the cephalic or saphenous veins. A constant dosage volume of 6 ml/kg body weight was used and dosages of 0.5, 2.0, 8.0 or 30.0 mg/kg/day were administered to the 4 treatment groups. The test material was dissolved with sterile physiological saline. The fifth group of animals (controls) received physiological saline.

Clinical signs and food consumption were monitored daily throughout the study. Body weight was determined weekly. Before, and durng weeks 6, 12 and 25 of treat-

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ment, the ESR, PCV, Hb, RBC, MCV, MCHC, reticulocyte count, total and differential leukocyte count, platelet count and prothrombin index, plasma glucose, serum urea, SAP, SAlT, SAsT, LAP, total protein with differential electrophoresis, bilirubin, sodium, potassium, inorganic phosphorus, chloride, calcium, cholesterol and creatinine were determined; 16 h urine samples were examined for volume, pH, specific gravity, proteins, glucose and reducing substances, ketones, bile pigments and haemoglobin, together with microscopic examination of sediment after centrifugation. For the 30.0 mg/kg/day and control groups these haematological and biochemical studies were performed also during week 3. Tests for faecal occult blood were made before dosing and during weeks 1, 2, 3, 4, 6, 12 and 25. Ophthalmoscopic examinations were made during the course of the study.

On completion of the dosing period, the animals were killed and subjected to post mortem examinaton which included weighing the brain, pituitary, heart, lungs, liver, spleen, pancreas, thymus, prostate or uterus, kidneys, adrenals and gonads, and a wide range of tissues were prepared for routine histological examination. Sections of liver, kidney and spleen were fixed in Karnovsky's fluid for subsequent preparation for electron-microscopic examination.

RESULTS

A female monkey at 30.0 mg/kg/day was killed for humane reasons on day 20 of treatment. A petechial rash first developed on the limbs on day 4; this persisted and became more extensive, covering the limbs, chest and face. Skin ulcers developed, which failed to heal. From day 15 there was a loss of appetite and a deterioration of physical condition. Blood samples showed an increased ESR, rouleaux formation and cell agglutination. The erythrocyte and platelet counts were reduced and the neutrophil count increased. Fibrinolytic activity was normal. Biochemical studies revealed reduced values for the serum protein. There was haematuria and the faeces contained occult blood. Macroscopically, apart from the multiple skin foci, the most significant findings were haemorrhages on the mucosal surface of the bladder, reddening of the duodenal mucosa, and congestion of the blood vessels of the cerebral hemispheres. The spleen was enlarged. A diagnosis of ulcerative dermatitis was confirmed histologically. All the other animals survived the dosing period.

Clinical signs were restricted to the skin and the visible mucous membrane. Most of the animals at 30.0 or 8.0 mg/kg/day developed rashes, which appeared first as a reddening of the skin with a few petechial haemorrhages. The rash usually occurred on the limbs, but sometimes the ears, face or tail were affected. In some animals ulcerative dermatitis occurred, and this was treated by application of an antiseptic dusting powder (10% sulphanilimide). One animal at 2.0 mg/kg/day developed minor skin lesions. Scleral haemorrhage was seen in 4 of the 5 animals at 30.0 mg/kg/day and in 2 of the 6 animals at 8.0 mg/kg/day. Nose bleeding, blood in the urine, and fresh blood in the faeces, was occasionally recorded from individual animals at 30.0, 8.0 or 2.0 mg/kg/day.

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TABLE I

TREATMENT-RELATED HAEM

Time of examination	Dose lentinan (mg/kg/day)	PC
Predosing (Mean ± SD) n = 30	0	47
6 weeks	Control	47
	0.5	46
	2.0	45
	8.0	44
	30.0	38
12 weeks	Control	48
	0.5	49
	2.0	48
	8.0	46
	30.0	42
25 weeks	Control	47
	0.5	47
	2.0	46
	8.0	43
	30.0	36

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Slight suppression of body weight gain was recorded in animals at 30.0 mg/kg/day accompanied by a reduction in food and water intake. Animals at 30.0 mg/kg/day and, to a less extent, animals at 8.0 mg/kg/day, became anaemic during the first weeks of the study, but the anaemia was not progressive (Table I). Examination of the red blood cells showed anisocytosis and hypochromasia, some rouleaux formation and in some animals, inclusion bodies, probably Howell–Jolly bodies. Reduced platelet counts were recorded and, by the end of the study, there was an increase in prothrombin time. No significant changes were found in the biochemical characteristics monitored. There was blood in the urine of some animals at 30.0 mg/kg/day.

At autopsy, widespread haemorrhagic discolouration and congestion involving the mucosa of the urinary bladder and all levels of the gastrointestinal tract were evident in animals at 30.0 or 8.0 mg/kg/day. Other organs affected were the liver, kidneys, thymus, testes, heart and skeletal muscle. Minimal congestion of the urinary bladder and duodenum was found in some animals at 2.0 mg/kg/day. There was enlargement of the spleen, liver and kidneys in animals at 30.0 mg/kg/day and, to a less extent, of the spleen and liver only, in animals at 8.0 mg/kg/day.

TABLE I

TREATMENT-RELATED HAEMATOLOGICAL CHANGES

Time of examination	Dose lentinan (mg/kg/day)	PCV	Hb	RBC	Platelets	Prothrombin index
Predosing (Mean ± SD) n = 30	0	47 ± 1.5	12.7 ± 0.58	5.1 ± 0.30	394 ± 74.7	98 ± 10.8
6 weeks	Control	47	12.1	5.6	284	100
	0.5	46	11.9	5.5	326	99
	2.0	45	11.7	5.5	231	101
	8.0	44	10.9	4.6	169	105
	30.0	38	9.0	3.6	101	98
12 weeks	Control	48	12.7	5.4	288	100
	0.5	49	12.9	5.7	357	101
	2.0	48	12.6	5.6	308	102
	8.0	46	11.7	4.8	251	105
	30.0	42	10.2	4.0	165	101
25 weeks	Control	47	12.8	4.4	303	101
	0.5	47	12.3	4.6	336	100
	2.0	46	12.0	4.8	282	101
	8.0	43	10.5	3.9	214	100
	30.0	36	8.2	3.5	124	93

Treatment-related morphological changes were seen in animals at 30.0, 8.0 or 2.0 mg/kg/day. In general, these were foam cell reactions in the lungs, liver, kidney, spleen, lymph nodes and bone marrow; with varying degrees of vasculitis in some tissues. The histopathological changes are summarised in Table II. The electron microscopic study of the liver, kidney and spleen showed the presence of inclusions in some cells. In the liver, and occasionally in the spleen, these inclusions had a filamentous appearance which was not evident in the kidney.

DISCUSSION

The effect of prolonged overdosage with lentinan has been determined in the rhesus monkey. The changes were similar to those found in the Beagle dog [2]. Two

TABLE II

SUMMARY OF HISTOPATHOLOGICAL CHANGES

	Daily dose (mg/kg)				
Change noted	0	0.5	2.0	8.0	30.0
Gastrointestinal					
Congestion of mucosa and especially duodenum	0/6	0/6	3/6	5/6	5/5
Petechial haemorrhages in oesophagus	0/6	0/6	0/6	4/6	4/5
Mucosal congestion in caecum and colon	1/6	1/6	0/6	4/6	4/5
Bladder					
Haemorrhage/congestion of mucosa	0/6	0/6	1/6	5/6	5/5
Subepithelial haemorrhage and/or vasculitis	0/6	0/6	1/6	3/6	5/5
Thymus					
Subcapsular petechial haemorrhages	0/6	0/6	0/6	1/6	2/5
Lung					
Foam cells in alveolar septum or lumen	0/6	0/6	0/6	3/6	5/5
Liver					
Intralobular and portal foam cells	0/6	0/6	6/6	6/6	5/5
Kidney					
Generalised foam cells	0/6	0/6	0/6	6/6	5/5
Granulomata/vasculitis	0/6	0/6	1/6	3/6	4/5
Lymphoreticular system					
Foamy macrophages in lymph nodes	0/6	0/6	2/6	5/6	5/5
Foamy macrophages in spleen	0/6	0/6	5/6	6/6	5/5
Foamy macrophages in bone marrow	0/6	0/6	0/6	0/6	5/5
Injection sites					
Perivenous accumulation of macrophages and multinucleate giant cells	0/6	0/6	3/6	6/6	5/5

significant effects were induced cell reaction and vasculitis. The cularly of the reticular endothel bodies were probably accumu macrophages and not indicative degrees of vasculitis and associa

In a study on fertility and ge dence of gonadal damage and i high dose levels. It must be note study were sexually immature a the testes.

In conclusion, it appears that monkey, reflects the administrat specific toxic manifestations. No tration of lentinan at 0.5 mg/kg

ACKNOWLEDGEMENTS

Thanks are due to Dr. Y. Sh pany, Tokyo, Japan, for supply

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se (mg/kg)			
0.5	2.0	8.0	30.0
0/6	3/6	5/6	5/5
0/6	0/6	4/6	4/5
1/6	0/6	4/6	4/5
0/6	1/6	5/6	5/5
0/6	1/6	3/6	5/5
0/6	0/6	1/6	2/5
0/6	0/6	3/6	5/5
0/6	6/6	6/6	5/5
0/6	0/6	6/6	5/5
0/6	1/6	3/6	4/5
0/6	2/6	5/6	5/5
0/6	5/6	6/6	5/5
0/6	0/6	0/6	5/5
0/6	3/6	6/6	5/5

significant effects were induced at 30.0, 8.0 or 2.0 mg/kg/day: viz. a general foam cell reaction and vasculitis. The foam cell reactions indicated overloading, particularly of the reticular endothelial system, with the polysaccharide. The inclusion bodies were probably accumulations of undigested polysaccharide within the macrophages and not indicative of a disturbance of lipid metabolism. The varying degrees of vasculitis and associated reactions accounted for the clinical signs.

In a study on fertility and general reproductive performance [3] in the rat, evidence of gonadal damage and impairment of reproductive capacity was found at high dose levels. It must be noted, however, that the rhesus monkeys used for this study were sexually immature and therefore with little or no active cell division in the testes.

In conclusion, it appears that the toxicological profile of Lentinan in the rhesus monkey, reflects the administration of large amounts of polysaccharide rather than specific toxic manifestations. No evidence was obtained to suggest that the administration of lentinan at 0.5 mg/kg/day induced clinical or histological changes.

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